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TRANSMISSION ELECTRON MICROSCOPY AT 2.5 MeV

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TRANSMISSION ELECTRON MICROSCOPY AT 2.5 MeV

Penetration and Reduction in Radiation Damage (Biological Specimens)

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ABSTRACT

Measurements of penetration on silicon and austenitic stainless steel have been continued using the same criteria as previously reported (G. Thomas, Phil. Mag., 17, 1097, 1968) up to 2.5 MeV using the 3MeV Toulouse electron microscope. The results show that penetration increases to about 14μ at 2.5 MeV for silicon although the curve starts to flatten out above 1.5 MeV, but no significant gain was found for stainless steel (~2μ at 2.5 MeV). Primary knock-on damage occurs readily in both materials. The critical voltages for 440 silicon and 422 tantalum were measured, and both found to be 1.4 MeV.

Measurements of radiation damage in ℓ-valine and glycine have shown very encouraging results, viz., a dramatic decrease in damage occurs above 1 MeV, contrary to what is predicted by "stopping power" theory. At 2.5 MeV the electron exposure that is tolerable is some 30 times greater than the critical exposure measured at 100 KeV. Thus, very high voltage electron microscopy is expected to be very significant for biological research, e.g. high resolution, and for observations of living cells as well as for research in the polymer field.

INTRODUCTION

Although the major advantages of high voltage electron microscopy have now received some considerable attention, so far experimental data have been mainly limited to up to 1.2 MeV, e.g. studies of penetration (Dupouy and Perrier 1962-64; Fujita et al. 1967; Thomas 1968; Hale and Henderson-Brown 1969; Uyeda and Nonoyama 1968; Humphries et al. 1971), critical (disappearance) voltage phenomena (Uyeda 1968; Bell 1969; Lally et al. 1970), radiation damage (e.g. Makin 1969; Urban and Wilkens 1972; Thomas et al. 1970; Kobayashi and Ohara 1966; Claffey and Parsons 1972; Glaeser 1971), improvements in resolution of bright field images of defects (Bell and Thomas 1972; Goringe et al. 1972), and applications in materials science and biology (for reviews see for example Dupouy 1968; Cosslett 1970; Bell and Thomas 1972; Fisher 1972).

In 1970 Dupouy et al. published their pioneering work describing the Toulouse 3 MeV microscope. It was anticipated that the advantages which could now be attained would include improvements in penetration. For metallic crystals primary knock-on damage should be observable in heavy metals and critical voltages attainable for a wide range of reflections and crystals. However, knock-on damage limits the applications of very high voltage electron microscopy of inorganic materials. Of significant interest is radiation damage in biological and polymeric specimens since this damage handicaps the attainment of high resolution images of single atoms and molecules, and dynamic observations of living cells. Although stopping power theories predict a decrease in specimen lifetime above 1 MeV, i.e., the radiation damage should not then decrease (e.g. Glaeser 1971), Cosslett (1971) suggested that the electron energy deposited in the sample is less at higher voltages so that a reduction

in radiation damage might then be expected. It is obvious that experimental data on this aspect are sorely needed.

Thus the present paper describes some results which have been obtained up to 2.5 MeV on the Toulouse microscope in which penetration measurements have been continued on Si and stainless steel using the same criteria as described previously (Thomas 1968), determinations of the disappearance voltage for the 440 reflection in silicon and 422 in tantalum were made and measurements of radiation damage in organic crystals (ℓ-valine, glycine) have been performed.

EXPERIMENTAL

Thick specimens of epitaxial silicon single crystals (diamond cubic structure) were especially grown for these experiments, so as to contain relatively high densities of stacking faults in order to facilitate the penetration measurements. Foils were prepared by chemical thinning in the usual way. Similarly foils of austenitic stainless steel (face centered cubic structure), which had been heat treated after deformation to contain twins and dislocation pile-ups, were obtained by electropolishing. Foils of silicon were also prepared from specimens which had been deformed at high temperatures.

Thin specimens of ℓ-valine and glycine suitable for radiation damage experiments were prepared as described by Glaeser (1971). The specimens 500 - 1000Å thick were mounted on thin carbon support films, and the damage determined by recording the lifetimes (times to destruction) of crystalline diffraction patterns as a function of beam current and accelerating voltage, e.g. Kobayashi and Ohara (1966); Glaeser (1971). The use of the diffraction pattern rather than the image, facilitates

measurements because shorter photographic exposure times are required.

RESULTS

a) Penetration

Figure 1 shows the results of the penetration measurements which were all carried out above 1 MeV using the same objective aperture size (20μ) with the foils oriented to excite a low order systematic set of reflections (220 for silicon, 111 for stainless steel). As in the previous experiments (Thomas 1968) the penetration limit was taken to occur at that thickness at which interference fringes at faults (Si) or twins(steel) were destroyed by absorption, care being taken to ensure proper focusing and maximum contrast at each stage of measurement. Since the Bragg angle changes with voltage, tilting is necessary to maintain optimum conditions. Typical examples of the diffracting conditions are shown in Figs. 2b and 3b. The foil thickness can be found accurately from the trace of the fault plane, foil orientation (from the Kikuchi pattern), and the tilt angles. Figure 2 shows an example of the method used: a) shows a stacking fault in silicon for which all fringes are visible at 2μ thickness at 1.5 MeV. The fault is then traversed into the thicker regions until the fringes are no longer visible (it is necessary to record these end points photographically) keeping the orientation constant. Magnifications used for obtaining the images were 5,000 x (accurate to 5%). In Fig. 2c there are still 6-8 fringes visible at 2.5 MeV at a thickness of 14μ (open circles, Fig. 1). Experience has shown that this condition corresponds to the useful practical limit for routine microstructural characterization. Whilst channelling can increase penetration, the resolution in the channelling mode is poor (Bell and Thomas 1972) and so

this orientation is not of significant practical interest.

Figure 3 shows an example of the thickness limit at 2.5 MeV for stainless steel. The twin fringes are damped out but the inclined dislocations still show about three oscillations at each foil surface. The resolution is good under these conditions, although screen visibility is poor and longer than normal exposure times (5-10 secs) are necessary to obtain such images. The thickness at this stage is about 2.2μ. It will be noted that there is no significant gain in penetration for stainless steel between 1 MeV and 2.5 MeV.* There is also no significant depth dependence of the resolution of the dislocation lines (top-bottom effect, Hashimoto 1964).

After these results were obtained, Humphries (1972) published a theoretical paper discussing the optimum orientations and voltages for penetration in metals taking aluminum, iron and gold as representative of light, medium and heavy elements. He suggested that 3 MeV is the maximum voltage for light elements, 2 MeV for medium weight metals and 1 MeV for heavy metals. These results are in general agreement with the present experimental data especially for light elements, although Humphries' theory appears to be more optimistic for medium weight metals than is actually observed (Fig. 1). However, at these high voltages knock-on damage and associated effects alter the microstructure e.g. formation of clustered point defects, Figs. 2,3, decoration of dislocations (Goringe et al. 1972), so that apart from investigations of radiation damage and its applications for reactor technology,

*This result has been confirmed using an independent method by Drs. Jouffrey and Reynaud at Toulouse.

accelerating voltages above one to two MeV are not very useful for metallurgical research.

b) Measurement of Critical Voltages

The critical voltages for 440 silicon and 422 tantalum were measured both by using the Kikuchi line and divergent beam techniques (Bell 1970; Lally et al. 1970) and the voltage calibrated by taking low magnification Kikuchi patterns (Thomas 1970). The values obtained were, coincidentally, the same viz., Si(440) 1.4 MeV, Ta(422) 1.4 MeV, to within experimental error (± 10kV).

c) Radiation Damage in Amino Acids

The specimens of &-valine and glycine are crystalline amino acids in which there is extensive hydrogen bonding between molecules. The principal damage occurs as a result of inelastic scattering in the sample and knock-on or displacement damage plays a relatively small role. Previous results (Glaeser 1971) indicated that the critical exposure that just corresponds to complete fading of the diffraction pattern is independent of the intensity (dose rate) of irradiation in the range 80 kV - 500 kV, indicating that specimen heating is not an important effect in the destruction process.

Figures 4, 5 show the results obtained at 0.5 meV and above. The current densities were determined directly with the use of a Faraday cage placed in the projector chamber, suitable allowance being made for the magnifications. A condenser aperture of 200μ was used and diffraction patterns recorded using the selected area diffraction mode. The data points in Figs. 4, 5 represent the average of at least six measurements. The fading times were all determined photographically as it was found

that the eye was not sensitive enough to determine the actual end of the destruction process and gives an underestimate of the lifetimes. A fine focus spot was used so as not to destroy too much of the sample on any one grid and the grids were scanned systematically from edge to edge. The data confirm that the lifetimes are independent of dose rate up to 2.5 MeV as the critical exposure plots all have slopes of minus one, on a log-log graph.

When the exposures are plotted (i.e. lifetime x current density) against voltage (Fig. 6), the great gain in lifetimes becomes obvious (e.g. a factor of ~ten from 1 MeV to 2.5 MeV or ~30 from 100 kV to 2.5 MeV, for valine). Also of great significance is that when the stopping power equation (Rohrlich and Carlsen 1954) is plotted it is immediately apparent that the latter simply does not apply and consequently much of the apprehension of biologists as to the usefulness of electron microscopy above 1 MeV has now been removed. A discussion of the further advantages in gain in resolution arising from the great decrease in damage rate with increasing energy will be given in another paper (Thomas and Glaeser 1972). It is suggested here that energies up to 5 MeV will be of great significance for biological and polymer research.

SUMMARY

Electron microscopy above 1 MeV has been shown to be extremely advantageous for organic/biological materials because of the great reduction in radiation damage. These encouraging results indicate that we may expect exciting developments in biological research e.g. a great gain in resolution and possibilities for direct studies of living cells.

In inorganic materials the gain in penetration appears to be significant only for light materials. However, critical voltages can be measured for a wider range of reflections and materials if a 3 MeV microscope is available.

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FIGURE CAPTIONS

Fig. 1. Penetration data obtained for silicon and stainless steel. Open circles correspond to thicknesses for which up to six fringes are visible at stacking faults. Closed triangles correspond to total loss of fringes. The open triangle at 2.5 MeV corresponds to the thickness for which all fringes are just visible. All results obtained for systematic reflections as shown in Figs. 2, 3.

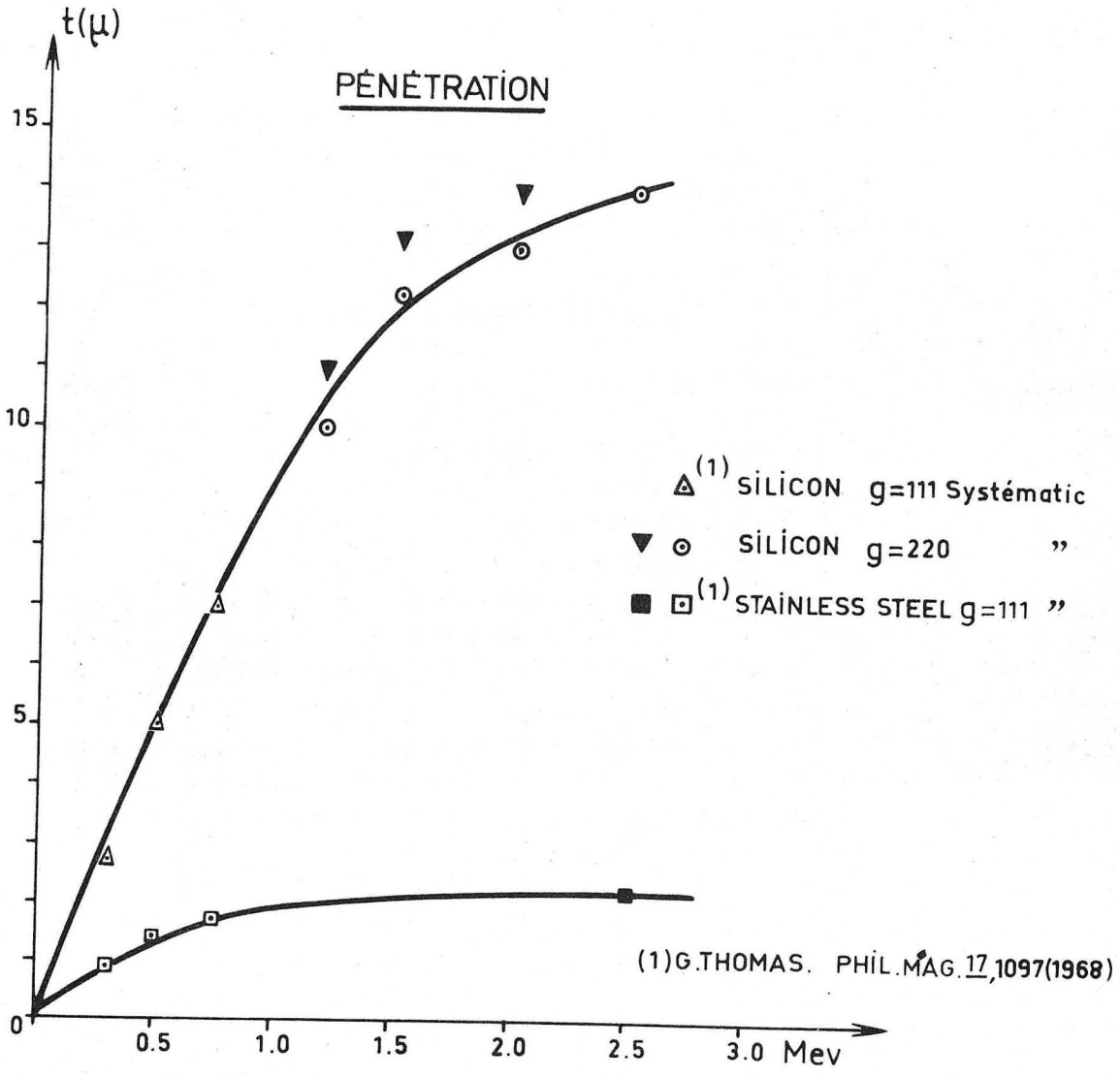
Fig. 2. Examples of the fringe technique for obtaining the data of Fig. 1. (b) shows the orientation of the systematic 220 set of reflections - bright field images a, c obtained for g = 220 with s slightly positive. Thicknesses obtained from the fault traces.

Fig. 3. Similar to Fig. 2 but for austenitic stainless steel. (a) bright field image g = 111 (s slightly positive). Notice good dislocation resolution but no fringe contrast at the twin interfaces; irradiation damage is evident. Thickness λ 2.2 μ at 2.5 MeV.

Fig. 4. Specimen lifetimes for complete destruction of diffraction patterns of ℓ-valine crystals as a function of beam current density and accelerating voltage.

Fig. 5. As fig. 4 but for glycine crystals.

Fig. 6. Critical exposure vs accelerating voltage for ℓ-valine and glycine crystals. The stopping power curve is also plotted (normalized to 0.5 MeV). The dramatic decrease in radiation damage with voltage above 1 MeV is apparent.



XBL 728-6771

Fig. 1

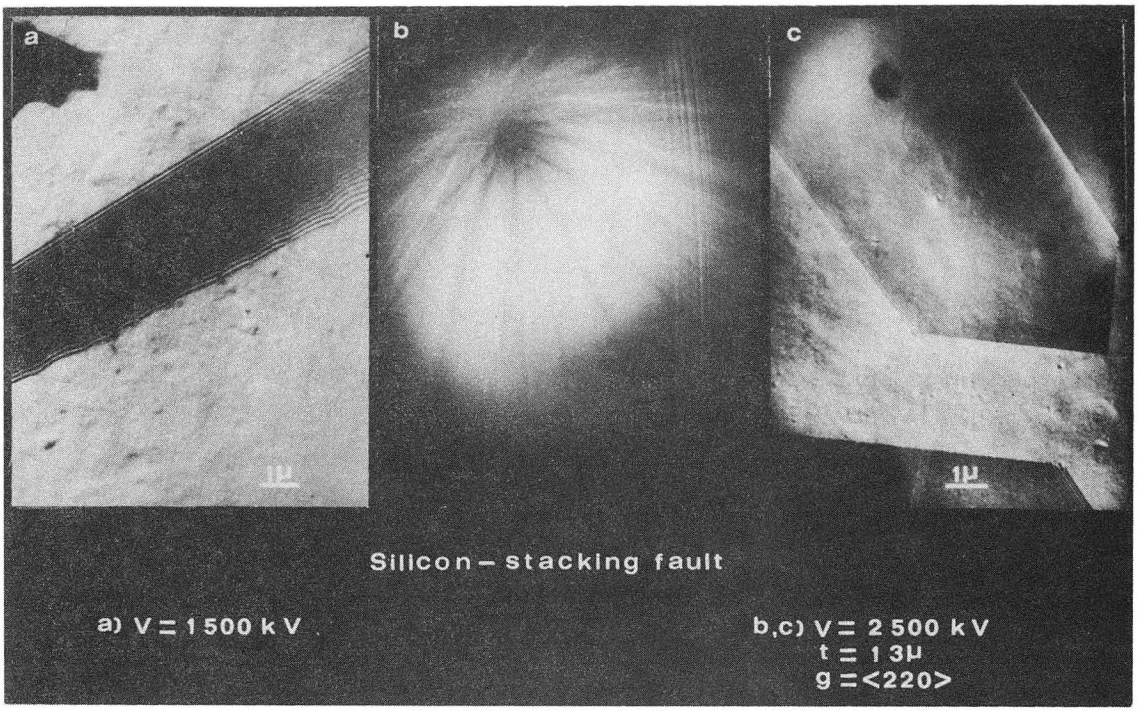


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Fig. 2

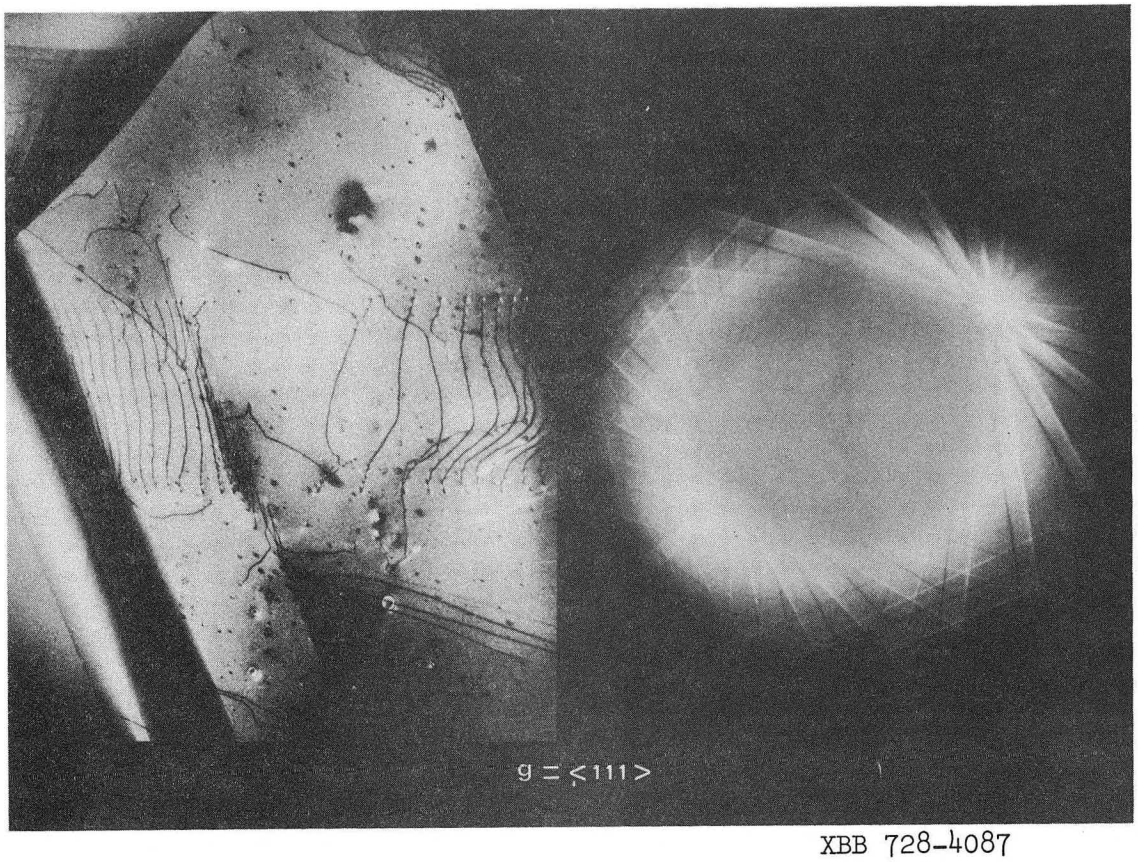

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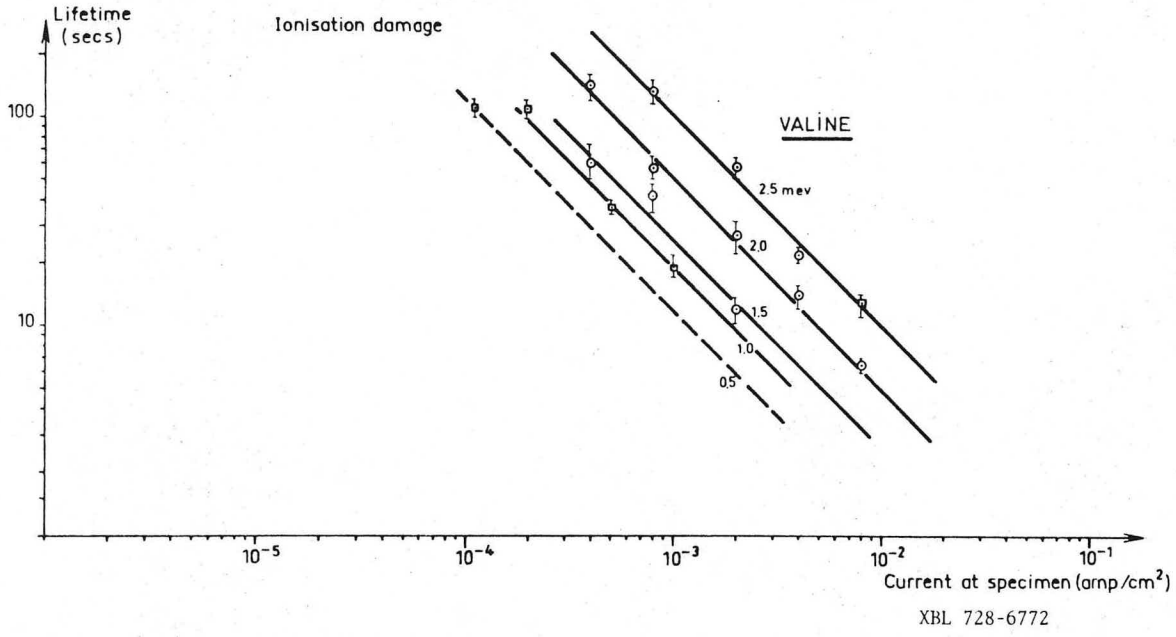
Fig. 3



XBL 728-6772

Fig. 4

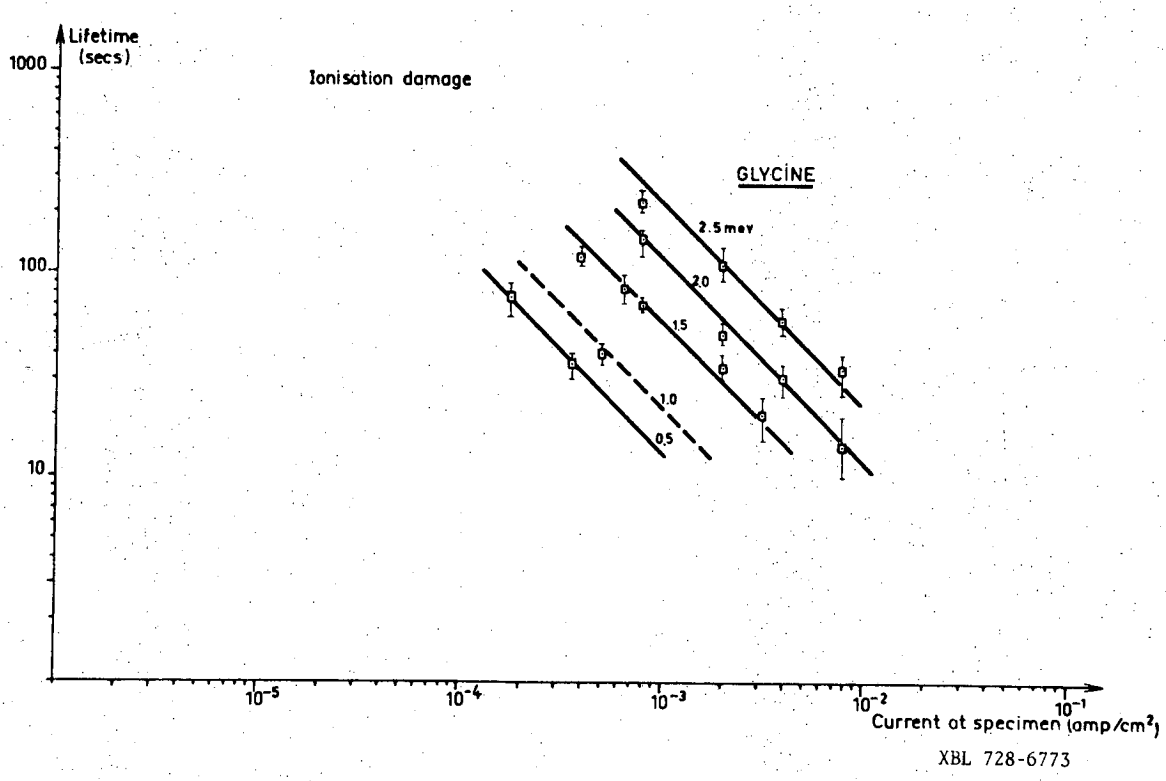

Lifetime (secs)
1000
100
10
Ionisation damage
GLYCINE
2.5 mev
2.0
1.5
1.0
0.5
10^{-5}
10^{-4}
10^{-3}
10^{-2}
10^{-1}
Current at specimen (amp/cm²)
XBL 728-6773

Fig. 5

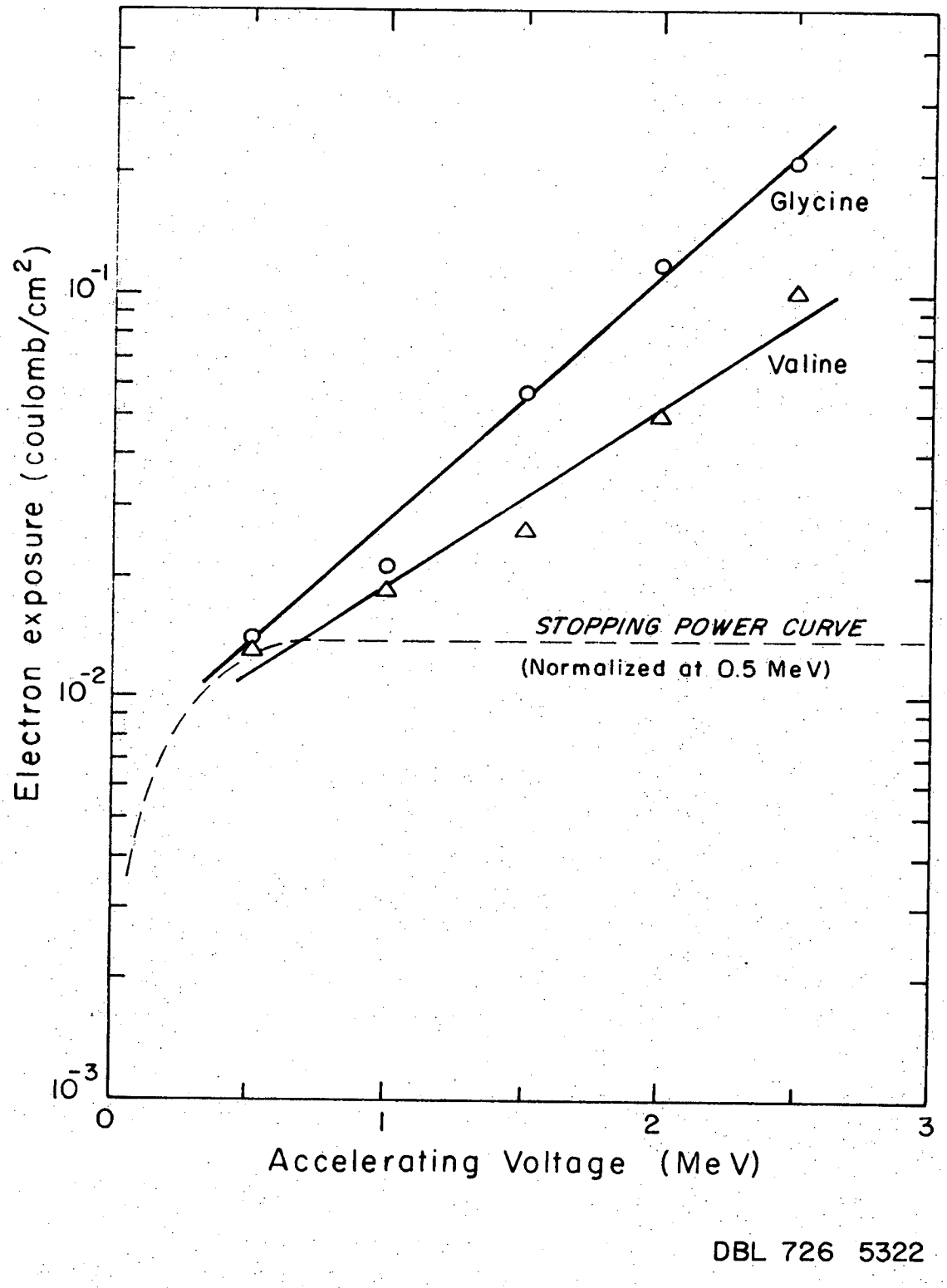

Glycine
Valine
STOPPING POWER CURVE
(Normalized at 0.5 MeV)
Electron exposure (coulomb/cm²)
10⁻¹
10⁻²
10⁻³
0
1
2
3
Accelerating Voltage (MeV)
DBL 726 5322

Fig. 6

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